A SPECIFIC ENZYME DEFECT IN GOUT ASSOCIATED WITH OVERPRODUCTION OF URIC ACID

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Gout is classed among the inborn errors of metabolism,[1] but unlike the majority of such disorders no single specific metabolic defect has yet been found to account for the hyperuricemia characteristic of this disease.[2] Although impaired renal excretion of uric acid is responsible for the hyperuricemia of some patients, an increased rate of purine biosynthesis *de novo* contributes to the hyperuricemia in the majority of gouty patients.[3] In affected members of certain families excessive production of purines is the sole cause of their hyperuricemia.[4]

Accelerated purine synthesis also occurs in other distinctive metabolic disorders.[5, 6] The increased rate of purine synthesis in patients with glycogen-storage disease (Type I) is attributed to the deficiency of glucose-6-phosphatase, which is the basis for this disorder.[5] We have recently demonstrated the complete absence (<0.05 % of normal) of an enzyme of purine metabolism, hypoxanthine-guanine phosphoribosyltransferase (HGPRT),[7] in four unrelated patients who have an X-linked familial neurological and behavioral disorder associated with excessive production of uric acid.[6, 8, 9]

Although no specific biochemical defect had been demonstrated to account for excessive uric acid production in typical gouty arthritis, a derangement in some aspect of the regulation of purine synthesis had been proposed.[10, 11] In this study we show that a partial loss of HGPRT activity is associated with excessive purine synthesis in some of these gouty patients.

Materials and Methods.—The rate of purine synthesis was evaluated in three ways. One method was to measure the uric acid excreted in a 24-hour urine after a patient had been maintained for at least five days on a diet essentially free of purines, containing 2600 calories, 70 grams of protein, 350 grams of carbohydrate, and 100 grams of fat. No drugs known to affect uric acid excretion or synthesis were administered. The average uric acid excretion over a three-day period for normal male subjects is 426 mg/24 hr (SD ± 81),[3] and excretion of quantities greater than 600 mg/24 hr was taken as evidence of excessive purine biosynthesis. Uric acid was determined in serum and urine by a specific method using uricase.[12] Urinary creatinine was determined[13] to assess the completeness of urine collections.

Purine synthesis was also evaluated in some patients by determining the pool size and turnover of uric acid by the use of isotopically labeled uric acid administered intravenously[14, 15] and by determining the extent of incorporation of isotopically labeled glycine into urinary uric acid.[16, 3]

Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (EC 2.4.2.8) and adenine phosphoribosyltransferase (APRT) (EC 2.4.2.7) activities were assayed by a radiochemical method. The reactions catalyzed by HGPRT and APRT are as follows:

$$\text{Hypoxanthine / Guanine} + \text{PRPP} \xrightarrow{\text{HGPRT}} \text{Inosinic Acid / Guanylic Acid} + \text{PPi}$$

$$\text{Adenine} + \text{PRPP} \xrightarrow{\text{APRT}} \text{Adenylic Acid} + \text{PPi}$$

Incubation mixtures contained 55 mM Tris buffer, pH 7.4, 5 mM $MgCl_2$, 1 mM 5-phosphoribosyl-1-pyrophosphate (PRPP) (Pabst Labs.), and 0.3 to 0.6 mg of protein from dialyzed erythrocyte or leukocyte lysates in a final volume of 100 μl. One purine base, 0.6 mM hypoxanthine-8-C^{14} (4.1 mc/mmole) (Calbiochem), 0.6 mM adenine-8-C^{14} (3.7 mc/mmole) (Volk Radiochemical Co.), or 0.14 mM guanine-8-C^{14} (12.6 mc/mmole) (Volk Radiochemical Co.), was present in the final reaction mixture. After incubation for 20 minutes at 38°C, the reactions were terminated by the addition of 2 μmoles of neutralized ethylenediaminetetraacetate (EDTA) and immediately frozen in a dry-ice acetone bath. Twenty μl of the reaction mixture was placed on 3MM Whatman paper with 0.06 μmole of the appropriate carrier nucleotide, and the reaction products were separated from substrates by high-voltage electrophoresis in 0.05 *M* borate buffer, pH 9.0, containing 0.001 *M* EDTA, at 4000 volts for 20 minutes. The area of paper containing the nucleotide product was then located by inspection of the paper under ultraviolet light or by autoradiography and cut out and counted in a liquid-scintillation counting system at 60 per cent efficiency. Nucleotide formation showed a linear relationship to incubation time and protein concentration under the conditions used. Erythrocytes used as a source of the enzymes were washed twice with two volumes of isotonic saline, hemolyzed by rapid freezing and thawing twice in a dry-ice acetone bath and then dialyzed for two hours in 0.001 *M* Tris buffer, pH 7.4, at 4°C. White blood cells were prepared by selective lysis of erythrocytes.[17] Leukocytes were lysed by freezing and thawing twice, using a dry-ice acetone bath.

Results and Discussion.—The marked decrease in activity of erythrocyte HGPRT found in five of fifteen gouty patients who produced excessive quantities of uric acid is shown in Table 1. The data presented in Table 2 indicate that the

TABLE 1

SPECIFIC ACTIVITY OF PHOSPHORIBOSYLTRANSFERASE OF ERYTHROCYTE HEMOLYSATES FROM NORMAL AND HYPERURICEMIC SUBJECTS

Subject	Urinary uric acid (mg/kg/24 hr) (mean)	Phosphoribosyltransferase Activity (mμmoles/mg protein/hr) Hypoxanthine (mean ± SD)	Guanine (mean ± SD)	Adenine (mean ± SD)
Normal (32)*	6.1	103 ± 18	103 ± 21	31.2 ± 6.0
Gout				
Normal uric acid production (6)*	6.1	99 ± 13	106 ± 10	31.2 ± 6.9
Excessive uric acid production				
Normal enzyme (10)* (range)	8.0 (4.4–16.8)	103 ± 18	104 ± 22	30.4 ± 5.3
Mutant enzyme				
J. family:				
F. J.	18.4	1.3	0.6	45.8
R. J.	15.8	1.5	0.8	43.2
T. J.	16.4	1.8	0.8	56.4
L. family:				
F. L.	23.9	11.8	0.5	74.0
M. L.	29.0	8.7	0.5	63.8

* Number in parentheses indicates number of subjects studied.

TABLE 2

SPECIFIC ACTIVITY OF PHOSPHORIBOSYLTRANSFERASE FROM LEUKOCYTE LYSATES

Subject	Phosphoribosyltransferase Activity (mμmoles/mg protein/hr)		
	Hypoxanthine	Guanine	Adenine
Normal, W. N. K.	128	183	211
Mutant, F. J.	8.7	6.1	237

enzyme deficiency is also demonstrable in leukocytes, showing that it is not confined to a single cell type. Hemolysate from affected patients added to the hemolysate of a normal subject did not alter the normal enzyme activity, suggesting that the presence of an inhibitor does not explain the decreased enzyme activity. The activity of the separate but closely related enzyme APRT was not diminished in these patients but was in fact moderately increased. The significance of this increase is being explored.

The five patients with HGPRT deficiencies were all males; all demonstrated a very high rate of uric acid production, and were members of two sibships. The three affected members of the J. family were aged 42, 49, and 55, and had had onset of acute gouty arthritis at ages 20, 33, and 31, respectively. Two of these brothers had a history of recurrent nephrolithiasis. The daily uric acid excretion of all three brothers was over twice that of normal,[3] and more detailed studies of two of the brothers showed a high rate of uric acid turnover and a degree of incorporation of labeled glycine into urinary uric acid that was over four times that found in normal subjects.[3, 4] They had no obvious neurological disorder and no disability other than that resulting from occasional episodes of acute gouty arthritis and complications from nephrolithiasis.

The two affected brothers of the L. family had a daily excretion of uric acid substantially higher than that of the J. family when related to body weight, and an incorporation of isotopically labeled glycine into urinary uric acid over ten times that found in control subjects.[18] The older brother, age 24, had had recurrent nephrolithiasis beginning at age 6 which lead to repeated pyelolithotomy and a right nephrectomy at age 10. Typical attacks of gouty arthritis began at age 13.[19] The younger brother, age 11, began having repeated episodes of hematuria at age 7, had documented nephrolithiasis and hyperuricemia at age 8, but has not yet developed clinical signs of gouty arthritis. Both brothers in the L. family had evidence of neurological disease characterized by spinocerebellar involvement which was not incapacitating and appeared to be quite distinct[18] from that found in children with the X-linked neurological and behavioral disorder.[6]

Most biochemical investigations of mammalian HGPRT have indicated that a single enzyme is responsible for the conversion of both hypoxanthine and guanine to their respective ribonucleotides. This view was supported by our finding that both activities were missing in children with the X-linked neurological disease and hyperuricemia.[7] In addition, we have been unable to separate the enzymatic activities for these purine substrates at pH 7.4 by heat inactivation, starch gel electrophoresis, gel filtration through Sephadex G-100, or partial purification.

The decrease in activity is the same within each family but differs between families. In the J. family the activity of the enzyme is 0.8 to 2 per cent of normal whether hypoxanthine or guanine is used as substrate. However, in the L. family there appears to be more activity when hypoxanthine is the substrate

(10% of normal) than when guanine is the substrate (0.5% of normal). Therefore, this is probably a reflection of some difference in the enzyme molecule rather than a disproportionate reduction in activity of two enzymes and suggests that the mutation leading to the loss of activity of this enzyme is different in each family.

As shown in Figure 1, heating at 80°C rapidly inactivates the erythrocyte HGPRT obtained from each affected member of the J. family, whereas the normal erythroctye HGPRT is considerably more resistant to denaturation at this temperature. In contrast, the mutant HGPRT from the two affected siblings of the

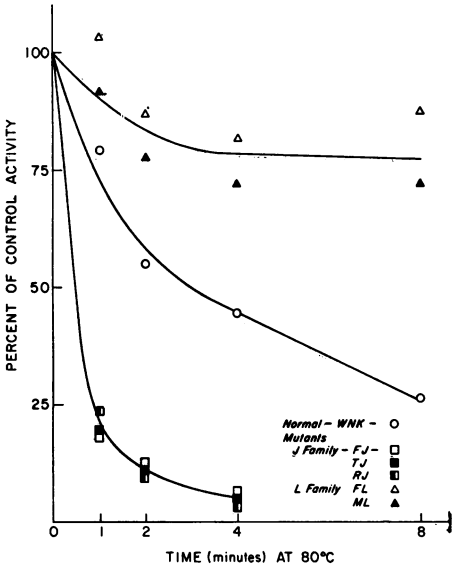

FIG. 1.—Heat stability of normal and mutant HGPRT. Dialyzed hemolysates diluted with 4 vol of 0.01 *M* Tris buffer pH 7.4 were heated at 80°C for the time indicated. After cooling, the normal enzyme was further diluted tenfold with 0.01 *M* Tris buffer pH 7.4 before assay, while the mutant enzymes were assayed without further dilution using 3 to 6 mg protein in the reaction mixture with incubation for 120 min.

J. family: F. J., NIH 00-92-19; R. J., NIH 02-29-33; T. J., NIH 03-04-04. L. family: F. L., NIH 07-24-96; M. L., NIH 07-24-97.

L. family is substantially more resistant to heat than the normal enzyme. A mixture of the dialyzed hemolysates from the J. and L. families showed the expected intermediate sensitivity of the HGPRT to heat, showing that no labilizing or stabilizing factors were present in the hemolysates. There was no dissociation of activity for hypoxanthine or guanine under these conditions with either the normal or mutant enzymes. These findings support the contention that the mutation and the resulting alteration of the enzyme molecule are unique to each family.

Several gouty patients who produce quantities of uric acid comparable to that of the J. family have normal HGPRT activity. This finding serves to emphasize that even the segment of the gouty population characterized by overproduction of uric acid is composed of a heterogeneous group of genetic abnormalities phenotypically expressed as gout. In addition this makes unlikely the possibility that the enzyme deficiency is a result of the overproduction of purines.

The association of excessive purine synthesis with a partial deficiency of HGPRT as well as with its complete absence[7] provides additional evidence that normal function of this enzyme is required for regulation of purine biosynthesis, although the precise mechanism by which the enzyme defect gives rise to overproduction of uric acid has yet to be determined.

The association in one family of a partial deficit of this enzyme with a mild neurological disorder apparently different from that found in children with the

X-linked familial neurological and behavioral disorder described by Lesch and Nyhan may be significant. Although the two neurological diseases have different clinical manifestations, the possibility remains that they may result from different degrees of the same basic abnormal process or its expression in different areas of the central nervous system.

Summary.—A partial deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase has been demonstrated in five members of two families having gout associated with overproduction of uric acid. The molecular alteration leading to the decreased activity of this enzyme appears to be different in each family. The mutant enzymes of the two families differ in their relative activity for each of the natural purine substrates as well as in their heat stability.

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